For Z-movement we have now used a faster piezo assembly with a resonant frequency of ~18 kHz, similar to the ones we used for hair bundle deflection. Despite having a less sensitive strain gauge sensor, the vertical resolution of the system remained the same (~5nm). After adjusting the proportional-integral-derivative controller of the Z-scanner (~50µs delay) and increasing the speed of approach, we were able to obtain high-resolution images of live hair cell bundles at a frame rate of 12 min/bundle or less.
We tested the performance of the improved HPSICM system in live rat inner hair cells (IHC) and showed, for the first time in live cells, the presence of characteristic stereocilia features at an X-Y resolution of ~11nm. We also imaged IHC bundles from the *Shaker2* and *Whirler* mice due to their short stereocilia with abundant stereocilia links (typically ~5nm in diameter and ~100-300nm in length). We confirmed the reproducibility of links in continuous time-lapse scanning and also their absence after chemical disruption with BAPTA-buffered Ca^{2+}-free medium.
Our results demonstrate that the improved HPSICM technique successfully visualizes the extremely convoluted surface of stereocilia in live auditory hair cells at a high resolution and a faster speed.
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4025-Pos Board B753
Compressed Sensing Based Atomic Force Microscopy
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The Atomic Force Microscope (AFM) is a powerful tool that has had a tremendous impact on the understanding of systems with nanometer-scale features. Efforts in improving the temporal resolution of the instrument have recently yielded high-speed AFMs with frame rates of approximately 10 frames per second. These instruments, however, achieve such speeds through various trade offs and as a result have limited imaging modes and scan sizes. In addition, despite these advances, typical commercial instruments continue to have frame rates well below one frame per second.
This work develops and implements a novel sensing matrix for the application of compressed sensing (CS) to image acquisition in AFM, with the goal of improving the temporal resolution of the instrument by reducing the amount of data that needs to be acquired to create a high-quality image. In traditional CS, each measurement is, by design, a linear combination of the elements of the signal under study. In AFM however, the physics of the sensing process require that each measurement contains information about only a single point. The CS measurement matrix used here takes this into account and allows the user to balance image acquisition time against image quality. The proposed method is demonstrated through simulation. These simulations show faithful recovery with a reduction in imaging on the order of a factor of ten. By accepting a reduction in image reconstruction quality, additional gains in imaging time, up to a factor of twenty, were achieved.

4026-Pos Board B754
A Novel Platform for Simultanoues Mechanical Stimulation and Characterization of Single Cells Based on Dielectric Elastomers and Atomic Force Microscopy
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We have developed a novel set-up to simultaneously 1) apply static and dynamic deformations to adherent cells in culture 2) optically image cells under fluorescence microscopy and 3) assay the near-membrane mechanical properties with atomic force microscopy. In this system, the cell culture substrate is formed by a film of dielectric elastomer which can be eletro-actuated. The geometry and position of the actuating electrodes and the applied potential can be manipulated to obtain specific strain fields over the cell culture chamber. We have modeled the electro-mechanical behavior of the actuated elastomer film and using optical markers we have established an experimental procedure to optimize and quantify the strain at the adherent cells. This cell culture device has been integrated together with a commercial atomic force microscope coupled with an inverted optical microscope equipped for fluorescence. This novel set-up allows us to temporally assess, with sub-micron spatial resolution, single cell topography and elasticity, as well as ion fluxes, all during static or cyclically applied deformations. Preliminary results on fibrobalsts (3T3 NIH) show reproducible and reversible increase in cell elastic modulus as a response to 4% applied uni-axial stretch; additionally high resolution elasticity maps of an area of 40x40 µm on a single fibroblast could be obtained while stretching a single cell. When measuring cardiomyocites from mouse embryo, profiles of Ca^{2+} intracellular concentration could be also monitored while applying static and dynamic stretches. This study provides proof-of-concept for this set-up as a flexible experimental platform to investigate mechano-transduction mechanisms at the single cell level.

4027-Pos Board B755
High Resolution Mass Spectrometric Imaging for Single Cell Metabolic Analysis
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Mass spectrometry imaging makes possible simultaneous measurement of a large number of biomolecules of lipids, peptides, proteins, etc. from the same sample. Of special interest are ambient ionization techniques that can be carried out at room temperature in air. We report the development of a new type of ambient mass spectrometry named laser desorption ionization droplet delivery mass spectrometry (LDIDD-MS). It utilizes a pulsed laser for desorption of molecules from cells or tissue substrates. The desorbed ions are picked up and delivered with directed sprayed liquid droplets on the laser-irradiated region to a mass spectrometer. By translating desorption region on XY moving stage, two-dimensional images of the desorbed/ionized ions can be formed. As the region of desorption/ionization of LDIDD-MS is spatially limited to the laser beam spot size, the spatial resolution can be ideally reduced to several microns.
We obtained spatial resolution as low as 2.4 µm in microcontacted standard samples and ~7 µm for a pancreas tissue sample. We employed the LDIDD-MS imaging for single cell analysis and observed a significant heterogeneity in cellular apoptosis of HEK cells. LDIDD-MS also enables real-time measurement/imaging of exocytosed biomolecules in live cells. Exocytosis of neuropeptides and enzymes in PC12 upon biochemical or biophysical stimulation has been acquired and we believe that this will make it possible for use to obtain spatiotemporally resolved maps of neurosecretions at single-cell resolution.

4028-Pos Board B756
Atomic Force Microscopy Reveals the Structure and Dynamics of the Cell Cortex
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Recent studies have shown that processes such as eukaryotic cell-cell interactions, differentiation and tissue development are controlled by mechanical signals. These mechanical stimuli come from the outside of the cells and induce remodeling of the cytoskeleton in the cell interior. Unfortunately, no dynamic data at high spatial resolution could be acquired so far on the cytoskeleton of live cells, and the mechanical heterogeneity at the subcellular level remains unknown. In particular, the cell cortex is a major determinant of the cell mechanics but its spatial arrangement is poorly understood, and the dynamic behavior of its elements could only be inferred through indirect methods. Here we demonstrate that simultaneous topography imaging and mechanical mapping of live cells under physiological conditions at high resolution and low forces is possible using atomic force microscopy. We applied our methods to perform direct imaging of the cell membrane actin cortex, reaching a resolution inferior to 100nm and a maximal 10s image acquisition rate. The cell cortex is structurally, mechanically and dynamically heterogeneous at the subcellular level, and its fastest rearrangement time was in the 10s range. Our resolution enabled direct sizing the sub-membrane actin meshwork, confirming estimates from electron microscopy and molecular diffusion studies. Furthermore, we can attribute dynamic parameters to actin meshworks of various architecture and estimate the forces that they can exert on neighboring cells.

4029-Pos Board B757
Nanopipet Based Nanoprobes for Single-Cell Analysis
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Molecular Biology has advanced our knowledge of the individual molecular components that make up living cells. The challenge, however, is to fully